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GENOME SEQUENCES

Isolation and Genomic Analysis of the Phage vB_PaeP_fHoPae04 Infecting Pseudomonas aeruginosa

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ABSTRACT Here, we report the genomic sequence of Pseudomonas aeruginosa phage vB_PaeP_fHoPae04, isolated from hospital wastewater in Helsinki, Finland. The phage genome is 45,491 bp long, has a G1C content of 52.2%, and contains 70 protein-coding genes and 3 tRNA genes.

Pseudomonas aeruginosa is a Gram-negative bacterium belonging to the ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) group of multidrug-resistant pathogens and is one of the major causes of nosocomial infections worldwide (1–3). As P. aeruginosa is associated with various diseases and is inherently resistant to a wide range of antimicrobials (4), it has become one of the most common targets for phage therapy (5).

Phage vB_PaeP_fHoPae04 (fHoPae04) was isolated from a hospital wastewater sample collected in Helsinki, Finland, using clinical P. aeruginosa strain 6886, isolated from a nasal sample from a chronic sinusitis patient, as the host. The strain was first incubated with the wastewater sample overnight, and the phage was isolated using three rounds of plaque purification as described elsewhere (6).

Phage DNA was isolated from a freshly prepared phage lysate with a phenol-chloroform extraction and ethanol precipitation (6), and next-generation sequencing was performed at Novogene (UK). The sequencing resulted in 9,833,124 150-bp fastq reads, out of which 100,000 reads were selected for assembly. The A5-miseq integrated pipeline version 0.7.5a-r405 for de novo assembly of microbial genome sequences was used to assemble the phage genome (7). PhageTerm (8) was used to estimate the genome termini, and the final assembly was verified by mapping the reads back to the genome using the Geneious Prime version 2020.1.2 Assembler and Find Repeats tool (Biomatters, Ltd.). All of the 9,833,124 original reads were used for the analysis with both PhageTerm and Geneious Assembler. The genome was annotated using the Rapid Annotations using Subsystems Technology (RAST) server (9–11), tRNAscan-SE version 2.0 (12, 13), BLASTP (14), and HHpred (15). CARD (16) was used to screen the phage genome for the presence of antibiotic resistance genes, and the BLASTN program (14) was used to identify the closest genome-wide relatives of the phage. Unless otherwise stated, default parameters were used for all software tools.

The fHoPae04 genome was 45,491 bp long with a G1C content of 52.2%. The median coverage depth was 309-fold. PhageTerm did not predict clear genome termini or terminal repeats, but 183-bp direct terminal repeats were identified with Find Repeats after the reads were mapped to the assembled genome sequence. The back-mapping resulted in a circular sequence, indicating that the assembled genome was complete.

Pseudomonas phages clash (GenBank accession number MT119362) and otherone (MT119373) were the closest relatives of fHoPae04 characterized so far, both having

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98% sequence coverage and 97.39% identity. This suggests that fHoPae04 belongs to the viral family Podoviridae and genus Bruynoghevirus.

The fHoPae04 genome had 70 protein-coding genes and 3 tRNA genes. Out of the 70 protein-coding genes, 49 were not assigned a function and were considered hypothetical. The 21 protein-coding genes having an identi able function included structural and assembly proteins (such as tail and capsid proteins and terminase subunits), proteins involved in DNA replication (DNA polymerase subunits, endo- and exonucleases, and helicase), and cell lysis (lysozyme). No genes related to the lysogenic life cycle or antibiotic resistance were identi ed, suggesting that fHoPae04 is lytic and suitable for phage therapy.

Data availability. The genomic sequence of vB_PaeP_fHo-Pae04 has been deposited in GenBank under the accession number MW329986. The associated BioProject, SRA, and BioSample accession numbers are PRJNA701388, SRR13694677, and SAMN17864917, respectively.

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REFERENCES

1. Obritsch MD, Fish DN, MacLaren R, Jung R. 2005. Nosocomial infections due to multidrug-resistant Pseudomonas aeruginosa: epidemiology and treatment options. Pharmacotherapy 25:1353–1364. https://doi.org/10.1592/phco.2005.25.10.1353.
2. Mielko KA, Jab onski SJ, Milczewska J, Sands D, ukaszewicz M, M ynarz P. 2019. Metabolomic studies of Pseudomonas aeruginosa. World J Microbiol Biotechnol 35:178. https://doi.org/10.1007/s11274-019-2739-1.
3. Pendleton JN, Gorman SP, Gilmore BF. 2013. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther 11:297–308. https://doi.org/10.1586/eri.13.12.
4. Livermore DM. 2002. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin Infect Dis 34:634–640. https://doi.org/10.1086/338782.
5. Pires DP, Vilas Boas D, Sillankorva S, Azeredo J. 2015. Phage therapy: a step forward in the treatment of Pseudomonas aeruginosa infections. J Virol 89:7449–7456. https://doi.org/10.1128/JVI.00385-15.
6. Sambrook J, Russel DW. 2001. Molecular cloning, a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
7. Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. https://doi.org/10.1093/bioinformatics/btu661.
8. Garneau JR, Depardieu F, Fortier L-C, Bikard D, Monot M. 2017. PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. Sci Rep 7:8292. https://doi.org/10.1038/s41598-017-07910-5.
9. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42:D206–D214. https://doi.org/10.1093/nar/gkt1226.
10. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.
11. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
12. Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44:W54–W57. https://doi.org/10.1093/nar/gkw413.
13. Chan PP, Lowe TM. 2019. tRNAscan-SE: searching for tRNA genes in genomic sequences. Methods Mol Biol 1962:1–14. https://doi.org/10.1007/978-1-4939-9173-0_1.
14. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. 2008. NCBI BLAST: a better Web interface. Nucleic Acids Res 36:W5–W9. https://doi.org/10.1093/nar/gkn201.
15. Zimmermann L, Stephens A, Nam S-Z, Rau D, Kübler J, Lozajic M, Gabler F, Söding J, Lupas AN, Alva V. 2018. A completely reimplemented MPI Bioinformatics Toolkit with a new HHpred server at its core. J Mol Biol 430:2237–2243. https://doi.org/10.1016/j.jmb.2017.12.007.
16. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen A-LV, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran H-K, Werfalli RE, Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-Koutoucheva A, Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D, Grif ths E, Maguire F, Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar GV, McArthur AG. 2020. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517–D525. https://doi.org/10.1093/nar/gkz935.